United States Department of Agriculture

Office of Agricultural Biotechnology

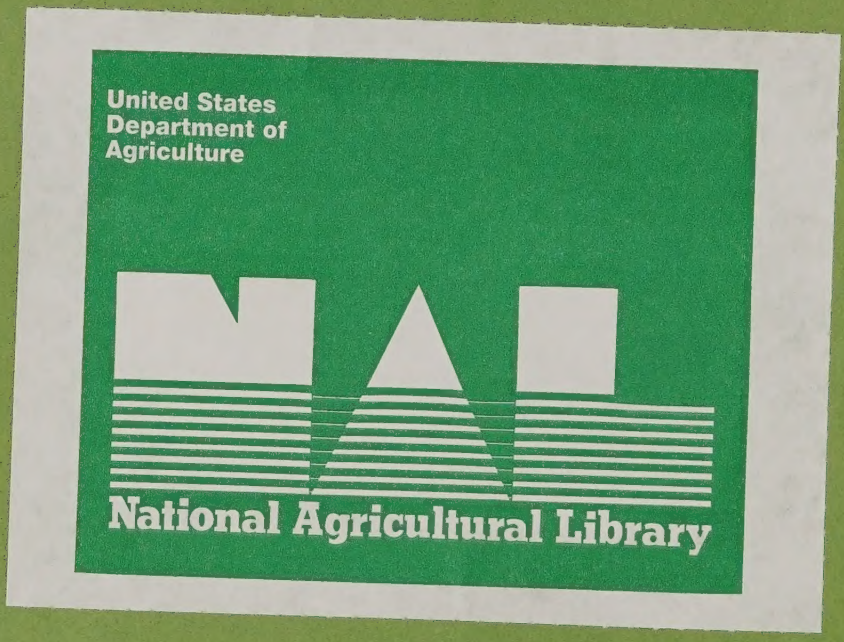
Minutes

Agricultural Biotechnology Research Advisory Committee

Transgenic Animal Working Group

April 8, 1993

Document No. 93 WG - 01


United States
Department of
Agriculture
National Agricultural Library

U.S. DEPARTMENT OF AGRICULTURE
Agricultural Biotechnology Research Advisory Committee
Transgenic Animal Working Group
Minutes of Meeting
April 8, 1993

The Agricultural Biotechnology Research Advisory Committee (ABRAC) Transgenic Animal Working Group (henceforth referred to as the Working Group) met on April 8, 1993, in Room 3109 of the South Building of the U.S. Department of Agriculture in Washington, DC. Dr. James Lauderdale chaired the meeting. The meeting was open to the public and had been announced in the *Federal Register* of February 11, 1993 (58 FR 8034).

Members of the Working Group in attendance were Dr. James Lauderdale, Dr. William Witt, Dr. Bennie Osburn, Dr. Susan Harlander, Dr. Ann Boyd, Dr. Richard Witter, Dr. Harold Hafs, Dr. Gary Weber, and Dr. Duane Kraemer. Persons in attendance from the Office of Agricultural Biotechnology (OAB) were Alvin L. Young, Maryln K. Cordle, and Barry Stone. Persons from the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) who attended were Dr. Marvin Norcross, Dr. Barbara Masters, Dr. Pat Basu, Dr. Oto Urban, and Dr. Bharat Patel. Others who attended are listed in Appendix A.

Call to Order and Preliminaries

Dr. Lauderdale called the meeting to order at 9:30 a.m. He introduced the members of the Working Group, and explained that the objective of the meeting was to develop a process that regulators can use to determine the safety of food from transgenic animals.

Dr. Young introduced the members of the OAB staff attending the meeting, and Dr. Norcross introduced the FSIS staff members who are involved in developing a transgenic animal policy.

Dr. Lauderdale asked Ms. Cordle to describe to the Working Group the events leading up to the meeting.

Background of Transgenic Animal Policy

Ms. Cordle explained that Dr. Norcross and FSIS staff had come before the ABRAC for assistance before. In June 1990, ABRAC was asked to review criteria developed by FSIS for evaluating the food safety of non-transgenic animals produced from transgenic animal experiments. These are animals for which the genetic transformation was unsuccessful -- the so-called, "no takes." The ABRAC reviewed and concurred in the proposed FSIS criteria. On December 27, 1991, FSIS published a notice entitled *Livestock*

and Poultry Connected with Biotechnology Research (56 FR 67054). This notice announced the availability of a document entitled *Decision Criteria for the Evaluation of Non-transgenic Animals from Transgenic Research* (attached as #191, Appendix B).

Ms. Cordle said that now ABRAC is being asked to address ways to evaluate the safety of food from transgenic animals. Specifically, FSIS wants the Working Group to address the food safety issues raised by transgenic animals and to answer the questions posed by FSIS in handout #190 (attached as Appendix C). She explained that other issues such as food labeling, societal concerns, animal health, and treatment of animals with bovine somatotropin are outside the scope of this meeting.

Ms. Cordle explained that the recommendations resulting from this meeting would be presented to the full ABRAC at its June 30, 1993 meeting in North Carolina. After that meeting, Dr. David Kline, chairman of the ABRAC, would submit ABRAC's recommendations to the Assistant Secretary of Agriculture for Science and Education, who in turn would forward the recommendations to the Assistant Secretary of Agriculture for Marketing and Inspection Services.

Dr. Lauderdale thanked Ms. Cordle and asked Dr. Norcross to describe the roles different Federal agencies play in regulating food safety.

Overview of Regulatory Roles

Dr. Norcross noted that a report by the General Accounting Office (GAO) had determined that 12 Federal agencies are involved with food safety.

Among those agencies, FSIS ensures that meat and poultry products are safe, wholesome, and accurately labeled, as specified by the Federal Meat Inspection Act (FMIA) and the Poultry Products Inspection Act (PPIA). Federal inspection is mandatory for the following animals used for human food: cattle, calves, swine, goats, sheep and lambs, horses and other equines, chickens, turkeys, ducks, geese, and guineas. FSIS annually inspects about 130 million meat animals and 7 billion birds at 7,000 plants (half are slaughter facilities; the other half are processing plants). Approximately 7,000 inspectors carry out this work, which includes inspection of each animal or bird at slaughter, and inspection of processed products during various stages of production.

The U.S. Food and Drug Administration (FDA) is responsible for assuring the safety of food not inspected by FSIS. FDA also ensures that animal drugs are safe (particularly with respect to residues present in the animal at slaughter), effective, and properly labeled. The Center for Veterinary Medicine, FDA, sets tolerances for residues in food. Both FDA and FSIS ensure that

food additives used in meat and poultry are safe for consumers, and the two agencies have a close working relationship.

USDA's Animal and Plant Health Inspection Service (APHIS) enforces the Virus Serum and Toxin Act and animal quarantine laws. Biologic products such as vaccines and serums used to protect the health of the animal are subject to APHIS oversight. The agency also enforces regulations to prevent the introduction or interstate spread of certain animal diseases or plant pests or diseases.

The Environmental Protection Agency (EPA) controls and abates pollution in air, water, solid waste, pesticides, radiation, and toxic substances. The agency also reviews and sets tolerances for residues of pesticide chemicals used directly on food animals or on animal feed crops before those chemicals are marketed and sets tolerances for the residues in food. Through a memorandum of understanding, USDA, FDA and EPA coordinate their activities in the regulation of food safety.

Dr. Young asked how fish are regulated. Ms. Cordle said that FDA is responsible for ensuring that fish and shellfish are safe for human food consumption.

Dr. Harlander asked if the six proposed questions in handout #190 came from FSIS alone, or reflected the consensus of several agencies. Dr. Norcross said that the questions had resulted from coordination with other agencies.

Dr. Norcross then asked Dr. Masters to discuss the proposed definition of transgenic animals and a possible approach to regulating them.

Approach to Regulating Transgenic Animals

Dr. Masters said that the proposed definition of transgenic animals is as follows:

> *Transgenic animals, for the purpose of this document, are animals whose genetic composition has been changed by introducing specific genes (e.g., recombinant DNA) from exogenous sources other than parental germplasm into the line from which the animals are derived.*

Dr. Masters noted that this definition is based on the perspective of a regulatory agency; it addresses food safety, not gene expression.

Dr. Masters also outlined an approach to evaluating the food safety of transgencic animals. The approach would consist of the following five steps:

1. Determining whether the animal is transgenic.

2. Determining whether the DNA is infectious. If it is, APHIS will have initial responsibility for regulating the animal.

3. Determining which group the transgenic animal belongs to. FSIS has proposed three groups: one for changes that relate specifically to the animal itself; one for somatic cell and other cell therapy; and one for bio-pharm animals. The first group would contain three sub-groups: changes that could be accomplished by traditional breeding; modification of existing traits considered unattainable by traditional breeding; and exotic gene transfers and new functions.

4. Review of the animal by the appropriate agency based on current regulatory authorities. Dr. Masters emphasized that no changes in regulatory authorities are anticipated.

5. Final food safety determination by FSIS.

Dr. Osburn noted that the definition proposed by FSIS addresses only the incorporation of DNA in the transgenic animal, but that FSIS's suggested animal groupings address the product of DNA expression. Dr. Masters explained that if the DNA is incorporated, FSIS will consider the animal to be transgenic. She added that the definition could be revised to incorporate the expression issue.

Dr. Boyd asked what level of DNA would be needed to determine whether an animal is transgenic. Dr. Masters replied that if the DNA cannot be detected by the most sensitive technology available, the animal is considered non-transgenic. Dr. Boyd then asked how the approach would deal with mobile genetic elements, and Dr. Masters asked the Working Group to help FSIS address that question.

Dr. Zimbelman asked whether the approach is limited to first-generation animals. Dr. Masters replied that the approach assumes that genetic transfer occurs from one generation to the next. Dr. Zimbelman suggested that the phrase "and subsequent generations" be added to the definition.

Dr. Kraemer noted that the definition may not need to distinguish parental germplasm.

Based on the foregoing, the following definition was accepted: "Transgenic animals are animals in which foreign DNA is detected."

Dr. Lauderdale suggested that the Working Group begin formulating the questions that regulators need for establishing a policy that

addresses the safety of food products derived from transgenic animals.

Questions Relating to Gene Products

The Working Group agreed that the first question to pose would be: "Is the animal in question transgenic?" The animal would be declared to be not transgenic based on the criteria described in the Federal Register notice of December 27, 1991 (56 FR 67054). Such non-transgenic animals would enter the human food chain without further action in accord with the provisions of that notice.

Animals would be declared to be transgenic based on at least one of the following: 1) detection of the transgene by Southern hybridization or polymerase chain reaction or other appropriate methods, or 2) presence of measurable gene product of the transgene, or 3) presence of transgene associated traits. The level of detection is defined as one copy of the foreign DNA per haploid genomic equivalent by DNA hybridization techniques.

The suggested approach for determining the human food safety of gene products is as follows:

1. Identify the gene product, the concentration of the product, and the tissue distribution (per edible tissue of concern in determining food safety).

2. If the gene product is endogenous:

    a. Review both the technical literature and regulatory history of that gene product and regulate based on existing human food safety guidelines.

    b. If there is no or limited technical literature and no or limited regulatory history for the gene product, convene an expert panel to define the assays, animal models, sensitivity, and specificity questions that must be addressed in order to determine human food safety.

3. If the gene product is non-endogenous, identify the expressed product. Questions posed should be based on the type of product identified.

    a. Perform assay validations for sensitivity and specificity with respect to food safety requirements.

    b. Identify the human food safety concerns associated with *in vivo* (surrogate and host animals) and *in vitro* assays using appropriate methodology. The unintended effect of a promoter-enhancer <u>cis</u> sequence used to express the desired gene should be tested *in vivo* and *in vitro* by

assays for the gene product. Secondary effects of enhancers on distant endogenous genes should be evident in model systems in order to predict deleterious side effects. The orientation of the promoter to the inserted gene during *in vitro* cloning is subject to experimental design. The cloning vectors have specific positions in which the gene and its regulatory elements are placed in ways that predict the successful expression of the gene product. However, enhancer elements influence gene expression from distal sites in both directions and in positive and negative ways in different cell types. Therefore, some testing strategy should be included in proposals that assay or detect unwanted effect of host genes.

c. Consult an expert panel to suggest methods and data requirements for any gene product that has too small a database to make a human food safety decision.

4. Determine secondary influences.

a. Determine if the DNA transferred is targeted to the gene insertion site since exogenous promoters and enhancers may have effects on near-by genetic sequences at or near the insertion locus.

b. Determine if there are any secondary effects (pleiotropic effects) of food safety concern related to the gene insertion site.

c. Determine if there are metabolic effects of the gene product, which are of human food safety concern.

The approach suggested above reflects the ideas of the Working Group members as well as those of other attendees.

Dr. Harlander questioned how one could scientifically detect the influence of one gene on other genes? Dr. Osburn suggested that the quantity of gene product would be a major factor.

A visitor, Dr. Judith Plesset, asked if marker genes should be addressed. Dr. Lauderdale replied that the expressed product of the marker gene must be identified. If it is a natural product (of the animal or a natural food product), then it can be examined in existing scientific literature and the human food safety determined. If it is not currently a gene product which is part of the food supply, then questions should be posed based on the type of product expressed, as determined in item 1 (identify the gene product). He then suggested that the product of the marker gene, rather than the gene itself, should be addressed. For that reason, item 3 was written as shown above, instead of specifically addressing marker genes.

Dr. Boyd suggested that this approach really encompasses two loops. The first loop addresses gene products that enhance something that is natural to the species and its secondary effects. The second loop addresses genes from another source, and asks about the metabolic and secondary effects of such genes. If a marker gene is included to track the exogenous recombinant DNA molecule, the marker gene and its product(s) is (are) subject to the same criteria as for transgenes because the marker gene is also foreign and presumably will be transferred with the exogenous elements into the host DNA.

The Working Group then began to develop an approach for evaluating the food safety of "naked" DNA.

"Naked" DNA

The Working Group agreed on the following approach for "naked" DNA:

1. Determine if the DNA is chemically integrated into the genome. If "Yes," then proceed with the gene products approach.

2. If naked DNA is used for vaccine or other purposes such as enclosure in synthetic vesicles or viral coats, then assays are needed to determine if the DNA remains in an "infectious" form. If "No," then the food product is safe. If "Yes," exclude until safety is determined by a panel of experts who would devise appropriate guidelines.

Chemically modified DNA, e.g., synthetic oligonucleotides with stabilizing modifications to nucleotides or the backbone, should be analyzed for a) stability and infectivity, and b) expression and then treated as above.

Dr. Lauderdale asked where "naked" DNA would come from. Dr. Osburn replied that it could come from liposomes or DNA guns.

Dr. Boyd asked how "naked" DNA could be dangerous to a food product. Dr. Osburn replied that it would be dangerous only if it recombined with another food agent. He noted that if bovine and human retroviruses recombine, it could become a food safety issue. Dr. Osburn also noted that the use of vaccinia vectors could pose food safety questions.

Dr. Kraemer suggested that the criteria used for detection of DNA could also be used to determine the disappearance time for the DNA.

Dr. Lauderdale noted that with the approach for "naked DNA" completed, the Working Group needed to be develop approaches for

somatic cells, mosaics, viral vectors, antisense, and amplifications and deletions.

Gene Amplifications and Deletions

Dr. Lauderdale suggested that questions regarding amplifications and deletions could be addressed by answering the questions developed for gene products. Dr. Boyd suggested that amplifications and deletions be considered independently only if gene insertion occurs at multiple locations, and if there are negative secondary effects.

Amplification of exogenous genes by use of expression vectors designed to include dihydrofolate reductase (DHFR) which amplifies during host DNA replication and thereby includes the foreign element in the amplification are used to insure enhanced gene expression. These designer vectors, therefore, should be tested for the level of product expression and for copy number by the same sensitive DNA hybridization techniques used in determining transgenics.

Deletions may occur through random illegitimate recombination of the foreign DNA into the host genome thereby inactivating a functioning allele. Such random integration is an expected effect of non-targeted gene therapy at the embryonic and somatic cell levels. Determination of the integration site against a known genomic background would help to predict gene "knock-out" effects and possible negative side-effects on the transgenic animal. Strategies for gene targeting at specific chromosomal loci are not currently expected in the production of transgenic animals. Therefore, one must assume that the norm is integration of foreign DNA at random positions and transgenic animals should thus be examined for any deleterious side effects of random integration.

The Working Group then began to develop an approach for evaluating viral vectors.

Viral Vectors

For viral vectors, the Working Group developed the following approach:

1. Viral vectors have been approved as safe for clinical trials in animals and humans in somatic cells, but not in embryonic tissue. Viral vectors are an alternative method of introducing foreign DNA into host cells in somatic therapy and in embryonic stem cells which, when incorporated into the developing embryo, result in chimeric or mosaic animals.

2. Viral vectors include a wide range of agents. Small DNA viruses whose insertion sequence along with viral DNA

integrates into the host chromosome without further virus replication should be treated as transgenics and evaluated in the same manner.

3. Cytoplasmic virus vectors, e.g., vaccinia, should be tested for appropriate expression of the exogenous gene product at levels appropriate for the gene product. The guidelines for safety should include determination of spread or infectivity of the virus and the effects of the gene product on the food product.

4. Retroviral vectors are designed to deliver foreign DNA into the host cell chromosomes. When used in embryonic tissue and integration occurs throughout the animal, the animal is by definition transgenic and should be regulated as such. When used in somatic cell therapy, the mosaic criteria should be applied. When specific proposals are submitted for use of a retroviral vector as a gene transfer agent, human food safety should be evaluated according to specific guidelines of an expert panel because recombination with endogenous viral sequences may require evaluative criteria in addition to those used for transgenics.

5. Viral enhancers have the potential to influence host cell genes near their insertion site and should be evaluated as stated for expression vector enhancers above.

Dr. Osburn expressed doubt that viral vectors currently in use pose problems to human health. However, vectored vaccines could pose such problems in the future, he added. He noted that bovine leukosis virus can infect human cells and produce secondary effects. Dr. Witter noted that retroviral "foreign" DNA occurs naturally in poultry.

The Working Group next addressed questions needed to evaluate the human food safety of mosaics.

Mosaic Animals

Dr. Boyd defined a mosaic as an animal in which a genetic transfer produces expression in some but not all of the animal's cells.

Dr. Lauderdale suggested that mosaics could be addressed directly by asking whether the animal is transgenic. The animal would not be considered transgenic if the inserted DNA were not detectable by the most sensitive technology available. A visitor, Dr. Gerald Messerschmidt, pointed out that because of the difficulty in getting DNA to incorporate beyond the first few cell divisions in many tissues, DNA can easily be detected using current technology. He also said that only the first generation of

mosaics — and a low percentage at that — are likely to be viable commercial products.

Another attendee pointed out that if the gene is incorporated into the germline of a a mosaic, the following generation would be transgenic. On the other hand, if the gene is not incorporated into the germline, the following generation would not be transgenic.

The Working Group recommended that the definition of a mosaic be an animal in which the foreign DNA has been inserted into some cells of the animal but not all. Throughout development, a mosaic animal has a "hybrid" character of being transgenic in selected tissues and not in others. Such animals may fail to test positive as transgenics since the foreign DNA is not present in all cells or tissues. Breeding of mosaics for several generations can produce occasional transgenics and, therefore, animals in subsequent generations should be tested for transgenic characteristics according to the criteria outlined above.

The Working Group then discussed the questions needed to evaluate antisense and somatic cell therapy.

Antisense and Somatic Cell Therapy

The Working Group agreed to the following approach in the case of antisense and somatic cell therapy:

1. The presence of the antisense gene in the animal should be determined according to criteria for detection of foreign DNA and the animal treated as transgenic if appropriate.

2. Expression of the antisense gene and specific target gene repression should be evaluated according the level of activity of the target gene product.

3. The transient therapeutic use of antisense therapy should be evaluated according to the specificity of the antisense genetic target's level of expression, time of effect, stability of the antisense nucleic acid, and the potential for integration. If not integrated, the agent should be evaluated according to the criteria for naked DNA or viral transfer and, if integrated, by the criteria for transgenic animals.

The Working Group discussed the methodological scope of the safety evaluation. Members agreed that the evaluation should address the safety of food products from organisms genetically modified by deliberate human intervention, but should exclude traditional animal breeding.

Dr. Lauderdale thanked the Working Group, and adjourned the meeting at 3:05 p.m.

Approved:

SUSAN McCULLOUGH
Rapporteur

DANIEL JONES
Editor

ALVIN YOUNG
Executive Secretary

JAMES LAUDERDALE
Chair

APPENDIX A

LIST OF VISITORS
UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE
TRANSGENIC ANIMAL WORKING GROUP
Meeting of April 8, 1993

Greg Riddle, Industrial Biotechnology Association
Gerald Messerschmidt, DNX Corporation
Judith Plesset, National Science Foundation
Linda Lee Bower, King Publishing
Ken Reid, *Food Chemical News*

John Matheson, Food and Drug Administration
Dennis Keefe, Food and Drug Administration
Pam Chamberlain, Food and Drug Administration
Richard Frahm, Cooperative State Research Service, USDA
James Maryanski, Food and Drug Administration

Robert Zimbelman, American Association of Animal Science
Gunnar Wilhelmsen, Embassy of Norway
Bob Frederick, Environmental Protection Agency
Althaea Langston, Animal and Plant Health Inspection Service, USDA
Jim Rasekh, Food Safety and Inspection Service, USDA

Bob Touverson, Food Safety and Inspection Service, USDA
John Mills, AFL-CIO
Ann Lichens-Park, Cooperative State Research Service, USDA
Dorothy D. Greenhouse, National Academy of Sciences
Bonnie Buntain, Extension Service, USDA

John Payne, Animal and Plant Health Inspection Service, USDA
Jay Blowers, Cooperative State Research Service, USDA

Appendix B

191

FEDERAL REGISTER NOTICE
67054

LIVESTOCK AND POULTRY CONNECTED WITH BIOTECHNOLOGY RESEARCH

FOOD SAFETY AND INSPECTION SERVICE
DECEMBER 27, 1991

Food Safety and Inspection Service

[Docket No. 90-025N]

Livestock and Poultry Connected With Biotechnology Research

AGENCY: Food Safety and Inspection Service, USDA.

ACTION: Notice.

SUMMARY: This notice (1) reaffirms a previously published policy statement concluding that animals involved in biotechnology experiments are research animals and are, therefore, subject to the existing regulations for livestock and poultry used for research, and (2) advises that the Food Safety and Inspection Service (FSIS) will inspect for food use livestock and poultry which were involved in such research but which are not genetically modified products of biotechnology. This notice is intended to accommodate public interest in Federal oversight of food applications of biotechnology.

EFFECTIVE DATE: December 27, 1991.

FOR FURTHER INFORMATION CONTACT: Dr. Marvin A. Norcross, Deputy Administrator, Science and Technology, Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, DC 20250, (202) 205-0495.

SUPPLEMENTARY INFORMATION: The livestock and poultry addressed by this notice are those derived from attempts

to bring about genetic improvements by transgenesis. Currently, this is usually done by injecting deoxyribonucleic acid (DNA) into fertilized eggs. Such experiments result in only a small proportion of animals being born with the intended genetic change. Currently, over 90 percent of the animals resulting from such experiments do not contain the intended gene. Because the genetic change is not present in these animals to distinguish them from the original animal line, they are not, in fact, transgenic.

In 1986, USDA published a statement of policy in the Federal Register (51 FR 23336) concerning research and the regulation of biotechnology applications in agriculture, including meat and poultry products. The policy statement (51 FR 23343) outlined how existing regulations would apply to food animals subjected to or resulting from biotechnology. It concluded that animals used in gene transfer experiments were subject to the experimental animal regulations, 9 CFR 308.17 and 9 CFR 381.75, because they were treated with a chemical such as injected DNA or other nucleic acid vector. This notice reaffirms that notice, specifically with regard to animals produced as a result of gene transfer experiments.

The experimental animal regulations specify that approval must be obtained from FSIS before experimental animals may be presented for slaughter under the Federal meat and poultry products inspection regulations. Thus, the sponsor or investigator of covered experiments must submit data or a summary evaluation of data to demonstrate that the products of livestock or poultry will not be adulterated. Points to consider in demonstrating that particular transgenic animal lines are not adulterated will be made available in 1992. For nontransgenic livestock or poultry derived from transgenic experiments, the data should be submitted to FSIS and would have to show that the animals to be slaughtered for food use do not have the experimental transgene and consequently are equivalent to the parental line and thus, are not adulterated as a result of the experiment. Given the current and continuously evolving technology in this area, there are likely to be a variety of ways in which to demonstrate that an intended genetic change has not occurred in the animals. The Agency will issue guidelines concerning what would be considered sufficient data, or an acceptable summary evaluation of such data. These guidelines will be provided by the Science and Technology Program, upon request.

Written approval of requests to present experimental animals for slaughter, if granted, will be provided by the Deputy Administrator, Inspection Operations. Upon presentation for Federal inspection of experimental animals that have been approved for slaughter, they would then be subjected to the same inspection procedures and regulations as food animals from traditional production practices.

Done at Washington, DC, on: November 12, 1991.

Ronald J. Prucha,

Acting Administrator

[FR Doc. 91-30980 Filed 12-26-91, 8:45 am]

BILLING CODE 3410-DM-M

DECISION CRITERIA FOR THE EVALUATION OF NONTRANSGENIC ANIMALS FROM TRANSGENIC ANIMAL RESEARCH

UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
SCIENCE AND TECHNOLOGY
WASHINGTON, DC 20250
(202) 720-8623

1991

Decision Criteria for the Evaluation of Nontransgenic Animals From Transgenic Animal Research

Connie L. Bacon, D.V.M., Residue Evaluation and Planning Division, U.S. Department of Agriculture, Food Safety and Inspection Service, 300 12th Street, S.W. Washington, DC 20250*

Tremendous advances have been made in livestock production in the last two decades. Artificial insemination and embryo transfer are now routine procedures in many cattle operations. Biotechnology promises even more advances in the production of livestock than artificial insemination or embryo transfer. In the near future it may be possible to develop commercially important changes in livestock in one generation that would have previously taken many years to accomplish through traditional selective breeding practices. Presumably the genetically engineered livestock of the future will provide producers with more efficient animals and consumers with products that were produced using fewer feed additives, are lower in fat and cholesterol and contain fewer pharmaceutical, biological and chemical residues.

In 1982, the first transgenic animals that expressed the injected foreign DNA were produced. These mice, containing a rat growth hormone gene linked to a

---

*Questions concerning the contents of this paper should be directed to Dr. Pat Basu, Director - Technology Transfer and Coordination Staff, USDA, FSIS, Rm 4911 South Bldg, Washington, D.C. 20250 or phone (202) 720-8623.

metallothionein promoter, are probably the best known transgenic animals produced to date. Thousands of transgenic mice have been produced since that time, with a wide variety of transgenes and for many different purposes. In 1985 the first attempts were made to develop transgenic livestock using the same methods employed in mice. The results were very disappointing and it is now recognized that the production of transgenic cattle, sheep, goats, and swine is far more difficult than the production of transgenic mice.

There appear to be four major reasons that transgenic livestock are so difficult to produce. First, in murine eggs the pronuclei are readily visible, making microinjection a fairly simple procedure. On the other hand, the cytoplasm of the larger mammals is very dense and special techniques must be employed in order to visualize the pronuclei. These techniques often reduce the viability of the embryos. Second, the recovery of suitable eggs for microinjection is more difficult. Fewer eggs per donor female are obtainable in livestock. Third, optimum *in vitro* culture conditions have yet to be established for pig, sheep, and cattle eggs through morula or blastocyst stages. The fourth factor that makes transgenic livestock more difficult to produce than mice is the long generational interval. The gestation of pigs, sheep, goats, and cattle range from 114 to 283 days with the onset of puberty ranging from six months to two years. This can be contrasted to the mouse which has a gestation of 20 days and a 28-day onset to puberty.

The efficiency of producing transgenic mice has been reported to approach 20% of the injected ova. The efficiency of producing transgenic livestock is less than 2%. Due to this extreme inefficiency and the economics of housing,

maintenance, and equipment required to produce transgenic livestock, it has been estimated that the average cost of producing a transgenic cow approaches $1 million. Therefore, due to the large number of nontransgenic animals which result from transgenic animal experiments and the high cost of maintaining these animals, it is not surprising that researchers are interested in slaughtering the nontransgenic animals which are a product of these types of experiments.

The production of mosaic transgenic animals is often a complication for researchers working with transgenic mice. Mosaics are animals which do not contain the transgene in all cells of the body. This is thought to occur in mice fairly often because of the timing of fertilization and microinjection of the one-cell embryo. It is hypothesized that the chromosomal DNA has already begun to replicate at the time of microinjection. In the production of transgenic livestock, the frequency of occurrence of mosaic animals is very low when one-cell embryos are microinjected. This is most likely due to the longer development time of the larger animals compared to mice. The DNA is not replicating as quickly in the larger mammals in this early stage and first cleavage occurs much later than it does in mice.

The mosaic mice which have been produced through microinjection techniques are uniformly mosaic in the somatic cells. Therefore, all organ systems possess approximately the same percentage of cells containing the transgene.

Mosaic transgenic animals can also be produced by using a viral vector as the means of introducing the transgene into an embryo which is usually at the 8-16

cell cleavage stage. Mosaicism in this situation is the desired outcome of the researcher and is a technique often employed to study gene expression.

A variety of methods are used to test the animals which result from transgenic animal experiments for the presence of the transgene. The two most commonly used methods are Southern Hybridization and the Polymerase Chain Reaction (PCR). Both of these methods can be highly specific and highly sensitive under the proper conditions. The sensitivity and specificity are based on the knowledge of the precise DNA sequence which was introduced into the embryo.

The amount of genomic DNA needed to generate a detectable hybridization signal using the Southern Hybridization method depends on a number of factors. These include the proportion of the genome that is complementary to the probe, the size of the probe and its specific activity, and the amount of genomic DNA transferred to the filter. Under optimum conditions, this method is capable of detecting 0.1 pg of DNA complementary to the probe with an autoradiographic exposure of several days. Therefore, a sequence 1000 bp in length that occurs only once in the genome (1 part in 3 million in mammals) can be detected on an overnight exposure, if 10 ug of genomic DNA is transferred to the filter and hybridized to a probe several hundred nucleotides in length. The use of PCR to amplify the gene sequence of interest allows the detection by Southern Hybridization of a single copy gene to be detected in the presence of a 10^{13}-fold excess of irrelevant DNA.

These two methods, Southern Hybridization and PCR, can then be used to test for the presence of the transgene in animals. Southern Hybridization and PCR

are also capable of detecting mosaic animals. These techniques are able to detect one copy of the transgene in 10% or fewer of the animal's cells. The most extreme mosaic reported to date is a mouse with the transgene present in 15% of its cells. The failure to detect the transgene by these methods supports the conclusion that the foreign DNA failed to incorporate into the genome of the animal.

The criteria that may be used to determine if the animals are nontransgenic are: 1) failure to detect the presence of the transgene by Southern Hybridization, the Polymerase Chain Reaction, or other appropriate scientific methods, 2) absence of a measurable gene product, 3) absence of transgene-associated traits, and 4) a healthy appearance.

The Food Safety Inspection Service (FSIS) has concluded that these nontransgenic animals (determined by some or all of the above methods) can be slaughtered safely under Title 9, Code of Federal Regulations (CFR) Sections 309.17, Livestock Used In Research. Animals exposed to a viral vector require prior approval by the Animal and Plant Health Inspection Service (APHIS). Under 9 CFR 309.17, the researcher must submit an application for slaughter to FSIS. This application must contain data demonstrating the methods employed to differentiate transgenic animals from nontransgenic animals. The application must also include such information as the number, age, sex, and identifying marks, such as tatoos or eartags, of the animals proposed for slaughter, and pharmaceuticals, biologics, or chemicals the animals were administered and the last date of administration, and the proposed date and establishment of slaughter. Upon receipt of all necessary information, FSIS

will review and evaluate the application. Provided that all criteria outlined in 9 CFR 309.17 are met, the animals described in the application will be approved for slaughter. These animals are identified upon presentation to the USDA Inspector-In-Charge at the slaughter plant as having been involved in research. They are maintained as a separate lot throughout the slaughter procedure. If the animals appear normal on antemortem and postmortem inspection they are passed for human consumption.

In addition to the research currently being conducted with transgenic livestock, there is also active research aimed at producing transgenic poultry. The production of transgenic poultry poses some unique problems for researchers, but the food safety of these birds would be assessed in a similar manner as transgenic livestock. If the birds are shown to be nontransgenic, then they may be eligible for slaughter under Title 9, CFR 381.75, Poultry Used in Research. The requirements of this regulation mirror those of 9 CFR 309.17.

FSIS feels that it is appropriate to slaughter these nontransgenic animals under 9 CFR 309.17 as livestock used in research or 9 CFR 381.75 as poultry used in research. On June 22, 1990, FSIS presented the proposed decision criteria for the slaughter of nontransgenic animals from transgenic animal research to USDA's Agricultural Biotechnology Research Advisory Committee (ABRAC). ABRAC unanimously voted to endorse the process by which FSIS will evaluate and present for slaughter such animals produced in the course of transgenic animal research.

Animals which are shown to contain the transgene by Southern Hybridization and/or the Polymerase Chain Reaction must be evaluated separately. FSIS is currently in the process of drafting a statement of the process which will be used to evaluate the food safety of meat, poultry, and meat and poultry products derived from products of biotechnology. FSIS plans to present this statement to ABRAC.

September 1990

Slightly revised December 1991, August 1992

§ 309.17 Livestock used for research.

(a) No livestock used in any research investigation involving an experimental biological product, drug, or chemical shall be eligible for slaughter at an official establishment unless:

(1) The operator of such establishment, the sponsor of the investigation, or the investigator has submitted to the Program, or the Veterinary Services unit of the Animal and Plant Health Inspection Service of the Department of Agriculture or to the Environmental Protection Agency or to the Food and Drug Administration of the Department of Health, Education, and Welfare, data or a summary evaluation of the data which demonstrates that the use of such biological product, drug, or chemical will not result in the products of such livestock being adulterated, and a Program employee has approved such slaughter;

(2) Written approval by the Deputy Administrator, Meat and Poultry Inspection Field Operations is furnished the area supervisor prior to the time of slaughter;

(3) In the case of an animal administered any unlicensed, experimental veterinary biologic product regulated under the Virus-Serum Toxin Act (21 U.S.C. 151 et seq.), the product was prepared and distributed in compliance with Part 103 of the regulations issued under said Act (part 103 of this title), and used in accordance with the labeling approved under said regulations;

(4) In the case of an animal administered any investigational drug regulated under the Federal Food, Drug, and Cosmetic Act, as amended (21 U.S.C. 301 et seq.), the drug was prepared and distributed in compliance with the applicable provisions of part 135 of the regulations issued under said Act (21 CFR part 135), and used in accordance with the labeling approved under said regulations;

(5) In the case of an animal subjected to any experimental economic poison under section 2(a) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (7 U.S.C. 135 et seq.), the product was prepared and distributed in accordance with § 362.17 of the regulations issued under said Act (7 CFR 362.17), and used in accordance with the labeling approved under said regulations.

(6) In the case of an animal administered or subjected to any substance that is a food additive or pesticide chemical under the Federal Food, Drug, and Cosmetic Act, supra, there has been compliance with all tolerance limitations established by said Act and the regulations promulgated thereunder (21 CFR 1.1 et seq.), and all other restrictions and requirements imposed by said Act and said regulations will be complied with at the time of slaughter.

(b) The inspector in charge may deny or withdraw the approval for slaughter of any livestock subject to the provision of this section when he deems it necessary to assure that all products prepared at the official establishment are free from adulteration.

§ 381.75 Poultry used for research.

(a) No poultry used in any research investigation involving an experimental biological product, drug, or chemical shall be eligible for slaughter at an official establishment unless the operator of such establishment, the sponsor of the investigation, or the investigator has submitted to the Inspection Service, or the Veterinary Biologics unit of Veterinary Services, Animal and Plant Health Inspection Service of the Department or the Environmental Protection Agency, or the Food and Drug Administration of the Department of Health, Education, and Welfare, data or a summary evaluation of the data which demonstrates that the use of such biological product, drug, or chemical will not result in the products of such poultry being adulterated, and the Administrator has approved such slaughter.

[37 FR 9706, May 16, 1972, as amended at 39 FR 4569, Feb. 5, 1974]

Appendix C

DRAFT

DRAFT 2/26/93

PRELIMINARY AGENDA
U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE
TRANSGENIC ANIMAL WORKING GROUP

April 8, 1993, 9:00 a.m. - 3:00 p.m.

Room 3109, South Building
U.S. Department of Agriculture
14th and Independence Avenue S.W.
Washington, DC 20250

(Times indicated are approximate and subject to change.)

1. Preliminaries

Call to Order (Dr. Lauderdale)	9:00 a.m.
Introduction of Members, Staff, and Visitors	9:05
Background and Charge to Working Group (Dr. Jones)	9:15
Approval of Agenda	9:25

2. History and Statutory Background of Federal Meat and Poultry Inspection (FSIS Staff) 9:30

3. Presentation of FSIS Proposed Policy on Transgenic Animals (FSIS Staff) 10:00

4. Working Group Discussion 11:00

Primary Reviewers:
- Dr. Boyd
- Dr. Osburn
- Dr. Witter
- Dr. Harlander

LUNCH ..... 12:00 noon

5. Comments of Visitors ..... 1:00 p.m.

6. Development of Working Group Recommendations 1:30

7. ADJOURN 3:00

FUTURE MEETING: Full ABRAC, June 30 - July 1, 1993
Research Triangle Park, NC

ABRAC TRANSGENIC ANIMAL WORKING GROUP

March 29, 1993

James Lauderdale, Ph.D., (Chair)
The Upjohn Company
7000 Portage Road
Kalamazoo, MI 49001
(616) 385-6573

William Witt, D.V.M., Ph.D.
National Center for Toxicological Research
Food and Drug Administration
NCTR Drive
Jefferson, AR 72079-9502
(501) 543-7949

Bennie I. Osburn, D.V.M., Ph.D.
Department of Veterinary Pathology
School of Veterinary Medicine
University of California, Davis
Davis, CA 95616
(916) 752-6865 & 7746

Susan Harlander, Ph.D.
Director, Dairy Foods Research and Development
Land O'Lakes, Inc.
P.O. Box 116
Minneapolis, MN 55440
(612) 481-2677

Ann Boyd, Ph.D.
Biology Department
Rosemont Avenue
Hood College
Frederick, MD 21701
(301) 696-3662

Richard L. Witter, D.V.M., Ph.D.
Avian Disease & Oncology Laboratory
Agricultural Research Service
3606 E. Mt. Hope Avenue
East Lansing, MI 48823
(517) 337-6828

Harold D. Hafs, Ph.D.
Animal Science Research and Development
Merck, Sharp & Dohme Research Laboratories
Rahway, NJ 07065
(908) 750-7454

Gary M. Weber, Ph.D.
USDA/Extension Service
Room 3334, South Building
14th and Independence Avenue S.W.
Washington, D.C. 20250-0900
(202) 720-2677

Duane C. Kraemer
Associate Dean for Research and Graduate Programs
College of Veterinary Medicine
Texas A&M University
College Station, TX 77843-4461
(409) 845-5092

U.S. DEPARTMENT OF AGRICULTURE
AG BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE
TRANSGENIC ANIMAL WORKING GROUP

WASHINGTON, D.C.
APRIL 8, 1993

FSIS PRESENTATION TO ABRAC TRANSGENIC ANIMAL WORKING GROUP
WASHINGTON, D.C. - APRIL 8, 1993

I. REGULATORY ROLES

The mission of the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) is to protect the public by ensuring that meat and poultry products are safe, wholesome, and accurately labeled.

FSIS enforces the **Federal Meat Inspection Act (FMIA) and the Poultry Products Inspection Act (PPIA).** Under these acts, FSIS inspects food-producing establishments to ensure that meat and poultry products sold in interstate and foreign commerce for human consumption are wholesome, unadulterated, and accurately labeled.
Federal inspection is mandatory for the following animals and birds used for human food: cattle, calves, swine, goats, sheep and lambs, horses and other equines, chickens, turkeys, ducks, geese, and guineas. The work includes inspection of each animal or bird at slaughter, and inspection of processed products during various stages of production.

The Food and Drug Administration (FDA) is responsible for assuring food safety from species other than those inspected by FSIS. FDA also assures animal drugs are safe, particularly with regard to the safety of residues remaining in the animal at slaughter, effective, and properly labeled. Therefore, FDA has authority over the regulation of any human or animal drug that may be derived from animal biotechnology under sections 502 and 512 of the Federal Food Drug & Cosmetic Act, and any human biologic product under section 351 of the Public Health Service Act. The FDA, along with FSIS, is charged with assuring that food additives used in meat and poultry products are safe for consumers.
The Animal and Plant Health Inspection Service (APHIS) is responsible for enforcing the Virus Serum and Toxin Act and animal quarantine laws. Biologic products such as vaccines and serums used in animal health are subject to oversight by APHIS for potential food safety impacts. Regulations to prevent the introduction or interstate spread of certain animal diseases or plant pests or diseases are also enforced by the service.

The Environmental Protection Agency (EPA) has as it's mission to control and abate pollution in the areas of air, water, solid wastes, pesticides, radiation, and toxic substances. Pesticide chemicals used directly on food animals or on animal feed crops are reviewed prior to marketing by the EPA.

II. TRANSGENIC LIVESTOCK

FSIS has not yet approved any transgenic livestock for slaughter. It is anticipated that each of these governmental agencies will be involved in regulatory issues pertaining to transgenic animals.

At the present time, there are no new laws, and there should not be any new laws, proposed for the slaughter of transgenic food animals. USDA's Coordinated Framework for the Regulation of Biotechnology (Federal Register Notice Vol. 51, No. 123, 23336, June 26, 1986) titled **"Final Policy Statement for Research and Regulation of Biotechnology Processes and Products"** states the Department's intention to regulate foods produced by new methods, such as recombinant DNA techniques, within the existing regulations.

Due to the low efficiency, there are considerable numbers of nontransgenic livestock produced from transgenic animal experiments. FSIS regulates the slaughter of these animals derived from biotechnology experiments under the experimental animal regulations as defined in 9 CFR 309.17 and 9 CFR 381.75. In December of 1991, FSIS published (Federal Register 67054-67055, Vol. 56, No. 249) A notice titled **"Livestock and Poultry Connected with Biotechnology Research".** This notice announced the availability of the document **"Decision Criteria for the Evaluation of Nontransgenic Animals from Transgenic Research."**

This document outlines the FSIS regulation for the slaughter of nontransgenic animals resulting from transgenic animal research the Decision Criteria document was reviewed by the APHIS, FDA, and was unanimously endorsed by USDA's Agricultural Biotechnology Research Advisory Committee (ABRAC).

III. CURRENT PLANS

FSIS is now at a point to seek guidance from ABRAC once again. This time, FSIS is seeking guidance on scientific issues related to food safety to help in the formation of their transgenic food animal policy.

Enclosed is the FSIS proposed definition of transgenic animal, a possible regulatory scheme, groupings of transgenic animals, and some questions pertinent to food safety of transgenic food animals.

FSIS has initiated this request for an ABRAC Working Group on the food safety of transgenic animals. FSIS has consulted with APHIS and FDA in the drafting of these questions that are being asked of the Working Group. These agencies will utilize scientific guidance gained from this meeting when working together to formulate their matrix of regulatory policies.

IV. FSIS PROPOSED DEFINITION FOR TRANSGENIC ANIMALS

Transgenic animals, for the purpose of this document, are animals whose genetic composition has been changed by introducing specific genes (e.g. recombinant DNA) from exogenous sources other than parental germplasm into the line from which the animals are derived.

V. POSSIBLE REGULATORY SCHEME

FSIS envisions a possible approach to the food safety evaluation of transgenic livestock and poultry as follows:

- o Is the animal transgenic?
- o Is the DNA infectious?
- o Groupings of transgenic animals
- o Review by appropriate agency based on current regulatory authorities of each agency
- o Final food safety determination by FSIS.

The exact regulatory scheme will be worked out by the involved agencies. At this time we are asking the ABRAC Working Group specific scientific questions that will be helpful to these agencies when forming the final regulatory sequence.

VI. POSSIBLE GROUPINGS OF TRANSGENIC ANIMALS

We have tried to envision the entire spectrum of transgenic animals and include them in a specific grouping. We have included the following groups:

- o Changes that relate specifically to the animal itself
  - changes that could have been accomplished by traditional breeding
  - modification of existing traits considered unattainable by traditional breeding
  - exotic gene transfers and new functions.
- o Somatic cell therapy
- o "Bio-Pharm" animals

We have tried to further define these groups as follows:

Group 1: Changes that relate specifically to the animal itself

- Change that could be accomplished by traditional breeding

Animals in this subgroup would include animals produced through recombinant DNA technology whose genomes are modified with genetic material from another strain of the same species. A major requirement for this classification is that the gene transfer occurs between two animals that could successfully interbreed naturally (e.g. Bos taurus and Bos indicus). This fact would override taxonomic classification when deciding if two animals are of the "same species".

In effect, these changes should be a more efficient way of introducing a desired trait (e.g., heat/cold tolerance, litter size) from one strain to another without the uncertain results of trying to introduce new traits through natural breeding.

Additionally, the concept of "same species" applies to the portion of the transferred DNA that is expressed. This distinction is made due to the fact that non-expressed portions of these gene transfers (e.g., promoter regions) may be of bacterial or other animal species origin.

- Modification of existing traits considered unattainable by traditional breeding

Animals assigned to this subgroup would be those that have had an autogenous trait or function modified to an extent considered unattainable by traditional breeding. Modifications in this subgroup still relate to only the genome of the target animal and a level of expression that is greater or lesser than could be ordinarily achieved by traditional breeding.

Examples of these types of changes include increase or decrease in production of endogenous hormones, enzymes or proteins through increased/decreased expression of the genes that normally regulate the production of these substances.

These types of changes could be achieved by introduction of antisense genes, gene amplifications and gene deletions.

(It should be noted that these changes may not fit within the FSIS proposed definition of transgenic animal, however we feel they need to be discussed to help us in future regulatory decisions.)

- Exotic gene transfers and new functions

Animals assigned to this subgroup would include recipients of genetic material that was obtained from another species with which it would ordinarily be unable to successfully interbreed.

Other animals assigned to this subgroup would be those whose metabolic or enzymatic machinery has been genetically altered such that the animal now possesses a new metabolic pathway. This may result in the production of new proteins in meat, milk, or eggs for enhanced disease resistance or increased animal production.

Another example would be an attenuated recombinant retrovirus that expresses thymosin and is used to generate germline transgenic pigs that are immunologically "superior" and shown to be resistant to clinical signs associated with pseudorabies virus infections.

Changes in this subgroup are aimed at modifying the growth characteristics and/or quality of food products derived from the target animal.

Group 2: Somatic cell and other cell therapy

Somatic cell therapy involves the insertion of genetic material into the cells of a particular organ of the body. The function of somatic cells are thereby changed and may then liberate a substance to prevent or control a common disease. For example, the intramammary infusion of genetic material to transform mammary gland cells to secrete antibacterial enzymes for the control of mastitis. Another example could be an attenuated recombinant retrovirus that expresses interleukin-2 and is used in the treatment of Tumor Infiltrative Lymphocytes extracted from porcine melanomas.

This group is analogous to cell therapy for humans, but directed for therapeutic purposes in animals.

(It should be noted that the animals in this group may not fit within the FSIS proposed definition of transgenic animal, however we feel they need to be discussed to help us in future regulatory decisions.)

Group 3: "Bio-Pharm Animals"

These animals have been genetically altered to manufacture a human or veterinary drug or biologic substance for commercial marketing. The substance is then harvested from the milk or blood of the animal. Examples include pigs that manufacture human hemoglobin, and goats and cattle that secrete pharmaceuticals or biologics into their milk.

VII. QUESTIONS

1a. Within each group, what information should be requested about the gene, regulatory elements, and gene product that can be used for a food safety determination? Will this information differ depending on the group?

1b. What other information might be utilized for a food safety evaluation within each group?

2. Overall, what concerns are there with the use of viral vectors, particularly in regard to food safety of the animals produced with this technique (e.g. retroviral vectors)?

3. Overall, what food safety concerns are related to the possible disruption of regulatory or coding sequences of host genes?

4a. Does this grouping scheme cover the entire range of transgenic animals?

4b. Are there other groupings that would be more appropriate for covering food safety risks?

4c. What specific information could be used to determine which animals fall into which group?

5. What are the possible food safety risks associated with each different group of transgenic animals?

6. Under Group 1, containing those changes related to the animal, how would the fine lines be drawn to determine the following:

- To what extent can a modification of an existing trait occur before this would be considered a new function?

- Which specific transgenic animals might fall into which subgrouping?